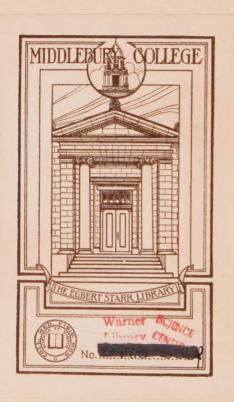
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# THE FROG A LABORATORY GUIDE

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By WALDO SHUMWAY

## THE FROG

A Laboratory Guide

## $\begin{array}{c} \text{BY} \\ \text{WALDO SHUMWAY, Ph.D.} \end{array}$

ASSOCIATE PROFESSOR OF ZOÖLOGY UNIVERSITY OF ILLINOIS

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#### PREFACE

The frog needs no introduction to the zoölogical laboratory, where it has assisted many generations of students in acquiring their first knowledge of vertebrate anatomy. As Professor Holmes writes in his Biology of the Frog, "They are nice clean animals, easy to dissect and admirably fitted for physiological experimentation."

In most localities they are easily collected and may be kept in the laboratory alive. All dealers in biological supplies handle them both alive and preserved. Natural sources of supply are being augmented constantly by the development of frog-hatcheries to supply the commercial demand.

The literature dealing with the frog is voluminous. No attempt is made in this guide to indicate more than a few of the books dealing with the anatomy of the frog which may be useful in supplementing this brief sketch.

It has been held by some that the function of a laboratory guide is to ask the student what he finds instead of telling him what he is to find, but, in the experience of many teachers, the "problem" type of laboratory manual is apt to waste the time of both instructor and student, and wear out the patience of both. It is the writer's opinion that the needs of the beginning anatomist are best served by presenting him with a straight-forward account of what he is to find in the specimen he is dissecting. If he encounters an anomaly or discovers the writer in a slip, he may be referred to larger manuals and to original contributions, and taste for himself the joys and hardships of research.

From time to time directions for the preparation of drawings are inserted. It is the writer's hope that these drawings will be used by the student as a means to the correlation of the anatomical details he has verified, rather than by the instructor as a method of judging the proficiency of the student. Frequent oral quizzes and demonstrations help make the laboratory a dissecting-room, instead of a drafting-room, as is too often the case.

Following the account of the anatomy of the adult frog, there will be found sections on the histology and embryology of this form. It is hoped that these will be useful in those laboratories where the frog plays the central rôle in the beginning course.

The terminology employed has been carefully compared with that of Holmes, which is followed as a general rule.

In conclusion, the writer wishes to express his appreciation of the kindness of his colleague, Dr. A. R. Cahn, in reading the manuscript and making many helpful suggestions.

WALDO SHUMWAY.

Urbana, Ill. April, 1928.

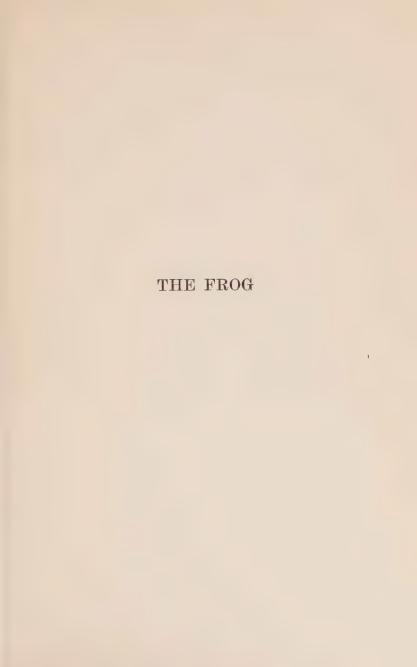
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### THE FROG

#### A LABORATORY GUIDE

#### INTRODUCTION

The frog is a tailless amphibian vertebrate. Its exact position in the system of classification is indicated below:

Phylum: Chordata, Sub-phylum: Vertebrata,

Super-class: Tetrapoda, Class: Amphibia,

Sub-class: Lissamphibia, Order: Anura,

Family: Ranidæ, Genus: Rana.

One hundred and ninety-five species of this genus have been described, twenty-one of which are native to North America. Of the latter the most common species is Rana pipiens, the leopard frog, upon the anatomy of which these directions are based. The largest species is the bullfrog, Rana catesbiana, and for this reason a more desirable form for laboratory work. The dissections here described have been repeated upon the bullfrog, and any striking divergences are noted. The latest key to the American species is contained in Boulenger's Monograph of the American Frogs of the Genus Rana. It should be noted that this author uses the specific name halecina instead of the

<sup>&</sup>lt;sup>1</sup>The tree frog and the toad belong to different genera although members of the same order.

more familiar pipiens. Excellent descriptions and illustrations are to be found in Miss Dickerson's Frog Book.

The following characters of the frog are peculiarly amphibian. The skin is glandular and without an exoskeleton. The skull is autostylic and its articulation with the vertebral column is by means of paired condyles on the exoccipitals. The basioccipital and supraoccipitals are lacking as well as the basisphenoid. The squamosals and parasphenoid are well-developed. The kidney is a mesonephros and there is present an urinary bladder. The male mesonephric duct functions as a ureter and gonoduct. In the female the mesonephric duct carries only excretory products, and an extra tube, the oviduct, is present to transport the products of the gonads. The heart has two auricles and one ventricle. Three aortic arches persist. The cardinal veins have been largely supplanted by a postcaval vein. Both a renoportal and an hepatoportal system are present. There is a comprehensive lymphatic system. The red blood corpuscles are large and oval with a prominent nucleus. The brain possesses a small cerebellum. Ten cranial nerves are found. The olfactory saes communicate with the mouth by means of posterior nares. There is no outer ear. The tympanic membrane is attached to a columella which extends across the middle ear to join a cartilage which has been considered the stapes, lying in the fenestra ovalis.

Gaupp's revision of Ecker and Wiedersheim's Die Anatomie des Frosches remains the most comprehensive memoir on the anatomy of the frog. The Biology of the Frog by Holmes is a convenient compendium with especial regard to R. pipiens.

The student must bear in mind that the frog is not a good example of the generalized amphibian type. Necturus, one of the tailed amphibia (see Adams, L. A., Necturus, a

Laboratory Manual) is preferable for this purpose. The Anura or tailless amphibia are characterized not only by the lack of a tail, but by the short broad trunk and the fact that the hind legs are much larger than the fore legs. The vertebræ are generally procoelous and the caudal vertebrae are fused into a single bone, the urostyle. No ribs are present. The skull shows certain peculiarities such as the fusion of the frontals and parietals, and the presence of a columella. A clavicle is present in the shoulder girdle, and the ilium of the pelvic girdle is greatly elongated. The radius and ulna are fused into a single bone, as are the tibia and fibula. In the respiratory system, no distinct trachea is found. The fifth aortic arch is absent in the adult. The kidney is shorter than in the Caudata.

The tissues of the frog are well adapted to serve as an introduction to histology. The cells are large while the organs are small enough so that sections through entire organs can be viewed within the field of the low power objective. Examples of all common vertebrate tissues may be found.

The embryology of the frog presents the following points of interest. Fertilization is external. The egg is fairly large and segmentation is quite regular through the first cleavages. Hemisections through the blastula and gastrula are instructive at low magnifications. The young hatch as larvæ (tadpoles) with first external and then internal gills. The large tadpole of the bullfrog is easily dissected. Stages in metamorphosis may be demonstrated.

Dissection is a technique which requires, above all things, both care and patience. Follow directions carefully. Never cut anything away without knowing what you are cutting and what you are looking for. If you get in trouble, stop, wash the specimen under the tap and make a fresh start. Keep your dissecting instruments clean and sharp. Clear

away the débris as it accumulates and put everything in its place at the end of the hour. Remember that accurate observation is as important as careful dissection. Do not depend on your memory, but make working sketches as you dissect. Do not neglect your ruler; a few accurate measurements will make your final drawing more valuable than hours of "finishing." Label every structure appearing on the drawing.

Learn the following descriptive adjectives: dorsal, with reference to the back; ventral, with reference to the belly; cephalic, with reference to the head (= anterior); caudal, with reference to the tail (= posterior); mesial, with reference to the mid-line; dextral, with reference to the right side of the animal itself; sinistral, with reference to the left side; proximal, referring to the part nearest the mid-line of the body; and distal, referring to the part farthest away from the mid-line. Any of these adjectives may become adverbs by the substitution of the suffix -ad for -al, e.g., dorsad means in the direction of, or toward, the back.

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#### SECTION I. EXTERNAL CHARACTERS

Place the frog on your dissecting pan, dorsal side up, and locate the following structures:

- 1. The head is roughly triangular in shape.
- 2. The mouth is large, terminal, and probably tightly closed.
- 3. The eyes are large and bulging. In the living specimen a central pupil, black and oval, surrounded by a bright, colored iris, can be distinguished.
- 4. There are two eyelids, upper and lower (the latter with an attached nictitating membrane which can with difficulty be demonstrated).
- 5. The brow spot, a light spot between the eyes, is the vestige of the pineal eye, and embryologically connected with the epiphysis (176).
- 6. Caudal to the eyes is the tympanic membrane (tympanum) of the ear, in the center of which is a slight projection caused by the columella (165), one of the ear bones. What significance do you find in its position on the tympanum?
- 7. At the cephalic end, the external (anterior) nares, or nostrils, are found.
- 8. The trunk bears two pairs of appendages.
- 9. The fore limb consists of upper arm, lower (fore) arm, and hand; the latter has four fingers and a vestigial thumb. The first and second fingers have three joints each, the others four.
- 10. The hind limb consists of thigh, shank, and foot; the latter has five toes and a rudimentary sixth

toe, the prehallux, on the inner side. The first two toes each have three joints, the next two have four, and the fifth, five. The toes are joined by a web. How far does it extend on each toe?

11. The dermal plicæ are folds extending caudad from the eyes. How many are there, and how far can you trace them on your specimen?

12. The vent or cloacal aperture is mesial and dorsal

to the hind legs.

Study the pigmentation. What colors are found, and where? What is the size, shape, and pattern of the spots? Both pigmentation and pattern are especially important in R. pipiens.

Make the following measurements, in millimeters:

- a) from snout to vent;
- b) head, length;
- c) head, width;
- d) snout;
- e) eye;
- f) width between eyes;
- g) tympanum;
- h) forelimb, and subdivisions;
- i) hindlimb, and subdivisions.

Drawing 1. Draw the dorsal view, the head towards the upper edge of the sheet, natural size (x1).1

Bullfrog (x½).

#### SECTION II. MOUTH AND PHARYNX

Open the mouth of the frog as widely as possible and locate the following structures, commencing with the roof.

13. The lips are thin and fleshy.

14. The maxillary teeth are on the mesial edge of the upper jaw; they are fine, conical, and closely set.

Note their extent.

15. The sulcus marginalis is a groove into which the lower jaw fits closely.

16. The vomerine teeth are in two groups at the ce-

phalic end of the roof.

17. The posterior (internal nares) are lateral to the vomerine teeth. Probe these from the external nares (7) with a bristle.

18. At the caudal end of the cavity close to the angle of the jaws, locate the openings of the Eustachian (auditory) tubes.

19. The portion of the cavity into which these open is

the pharynx.

20. The pharynx communicates with the digestive tract by the œsophagus, a transverse slit in the mesial line, between the Eustachian tubes.

21. Cephalad to the œsophagus and on the ventral surface of the pharynx is a cephalo-caudal slit, the glottis, opening into the larynx (in reality, laryngeotrachea) (60).

22. The glottis is surrounded by two prominences caused

by the arytenoid cartilages of the larynx.

- 23. Lateral to the glottis, near the angle of the jaws, in positions corresponding to the Eustachian tubes, may be found the apertures of the vocal sacs if the specimen be a male. These may be probed or demonstrated with the blow pipe in a fresh specimen.
- 24. The tongue, large and fleshy, is wholly in the mouth cavity. Examine the peculiar method of its attachment.

Drawing 2. Draw (x2) a full front view of the mouth and pharynx, dorsal side uppermost, the jaws opened wide so as to show the buccal structures.

#### SECTION III. THE VISCERAL CAVITY

#### 1. THE VISCERA UNDISTURBED

Place the frog on the dissecting board, ventral side up, and with the head away from you. Holding the skin away from the underlying body wall with forceps, make an incision through the skin only from the pelvic girdle to the lower jaw. Carefully lift the skin with forceps and note the following.

- 25. Subcutaneous lymph spaces are separated by the points of attachment between the body wall and the skin. In life they are filled with a colorless fluid, the lymph.
- 26. On the inner surface of the skin are the cutaneous arteries and veins, by means of which cutaneous respiration is performed. Make out the points at which these enter the underlying tissues.
- 27. There is an abdominal vein, showing through the muscles of the body wall on the mid-line.

Cut through the body wall slightly to one side of the abdominal vein. Lift the body wall away from the underlying organs with your forceps and take care not to cut across any blood vessels. Use heavy scissors to cut across the pectoral girdle. Complete this incision to the same limits attained in the skin incision. Note the following structures without disturbing their position.

28. At the cephalic end is the heart, underneath the pectoral girdle.

- 29. It lies in the pericardial cavity, which is surrounded by
- **30.** a thin glistening membrane, the **pericardium**, which separates the pericardial cavity from
- 31. the abdominal cavity, or coelome, which contains the remaining viscera.
- 32. This cavity is lined by a membrane which continues over the viscera and is called the peritoneum.
- 33. The most conspicuous organ of the viscera is the liver, a large, dark, reddish brown gland just caudal to the heart. Between its two lobes
- 34. the dark green gall bladder may sometimes be seen.
- 35. Caudal to the liver and partially concealed by its lobes is the large, white, U-shaped stomach.
- 36. Sometimes the pancreas, a yellow, flat, and elongated gland with irregular lobes, may be seen at the point where the stomach joins the
- **37.** duodenum, or cephalic portion of the intestine, not always visible when the organs are undisturbed.
- 38. The urinary bladder is very large and has a thin wall. It is best demonstrated in a fresh specimen by inserting a blowpipe into the vent and distending it with air.
- 39. The large ovaries filled with black and white eggs are the most prominent structures visible in a mature female at certain seasons of the year.
- Drawing 3. Make a sketch (x1) of the viscera as they lie in place without disturbing them. If your specimen is a mature female, turn to page 18 and work out the structure of the female reproductive system before continuing.

#### 2. THE DIGESTIVE ORGANS

Push a needle handle or pipette into the opening of the asophagus (20), if possible as far as the stomach (35), and raise the liver, without cutting anything. Trace the entire digestive system, noting that it is essentially a tube, or canal with an anterior and posterior aperture, and having two large glands, the liver and pancreas, emptying into it. Locate the following structures which have not been previously noted:

- 40. The cardiac end of the stomach is that into which the œsophagus enters.
- 41. The posterior or pyloric end is where the stomach empties into the duodenum (37).
- 42. The bile duct can be demonstrated in a fresh specimen by squeezing the gall bladder (34) gently. From the gall bladder it empties into the duodenum a short distance below the pyloric end of the stomach. It is formed by the union of the
- 43. hepatic ducts, leading from the lobes of the liver and
- 44. is connected with the gall bladder by the cystic ducts.
- **45.** The ducts of the pancreas (36) open into the bile duct but are not easily demonstrated.
- **46.** The duodenum continues caudal to the point where the bile duct enters as the small intestine or ileum.
- 47. The ileum terminates in the large intestine or rectum, which enters
- 48. the cloaca, the external aperture of which is the vent (12).

#### 3. The Mesenteries

You should have noted by this time that the viscera are attached to the body wall as well as to each other by delicate membranes. These are the mesenteries. Locate the following membranes.

- 49. The mesogaster extends from the stomach to the dorsal body wall.
- 50. The mesoduodenum stretches from the duodenum to the dorsal body wall.
- 51. The mesentery proper is the membrane from the small intestine to the dorsal body wall.
- 52. The mesorectum is a corresponding structure from the rectum to the dorsal body wall.
- 53. The gastro-hepatic omentum stretches from the stomach to the liver.
- 54. The gastro-duodenal omentum extends from the stomach to the duodenum.
- 55. The hepato-duodenal omentum connects the liver and the duodenum.
- 56. The ventral ligament (mesohepar) is a portion of the embryonic ventral mesentery from the liver to the ventral body wall.
- 57. The spleen is a small dark red hæmolymph gland in the mesentery proper.

Drawing 4. Make a semi-diagrammatic sketch of the digestive system (x1), including the mesenteries.

#### 4. THE RESPIRATORY SYSTEM

The larval frog, or tadpole, breathes by means of gills. The adult possesses functional lungs, but also respires through the skin by means of the cutaneous veins and arteries (26).

Lift the liver and observe:

- 58. The lungs are brown, more or less shriveled structures at the cephalic end of the abdominal cavity. In the fresh specimen they may be demonstrated by inserting the blow pipe into the glottis (21) and inflating them. The blood vessels then apparent are
- 59. the pulmonary arteries and veins.
- 60. The tube leading from the glottis to the lungs is the larynx. Probe it.

Add the lungs to Drawing 4, and show the larynx by dotted lines.

#### 5. The Visceral Arteries

The digestive system must be removed in order to see the underlying structures more clearly. But before doing this it is advisable to identify the arteries and veins associated with these structures. In an uninjected specimen the arteries, carrying blood away from the heart, are usually empty and colorless, while the veins, which carry blood toward the heart, are congested and therefore reddish brown in color. In singly injected specimens the arteries are filled with a colored starch mass, while the veins are filled with blood. In doubly injected specimens, the portal systems (76-80) are injected with a contrasting color mass. Triply injected frogs have a mass of a third color injected into the remaining veins.

Identify the following arteries without cutting anything.

61. Tip the liver to one side and lift the stomach, turning it to your left, and in the mesentery proper (51) in front of the spleen (57) observe the cœliacomesenteric artery. It arises from the dorsal aorta (139) and soon divides into two branches (62 and 69).

- 62. The cephalic branch is the cœliac artery. It divides into two branches (63 and 64).
- 63. The left gastric artery runs along the mesogaster (49) sending numerous branches to the left side of the stomach.
- 64. The gastro-hepatic artery runs from the cœliac, after the left gastric has been given off, along the mesoduodenum (50) and divides into two branches (65 and 67).
- 65. The right gastric artery runs from the gastrohepatic along the gastroduodenal omentum (54) to the right side of the stomach which it supplies by many small branches. It gives off in passing
- 66. a small gastro-pancreatic artery.
- 67. The hepatic artery, a branch of the gastro-hepatic (64), runs along the hepato-duodenal omentum to the liver, which it supplies with arterial blood.
- **68.** A small hepato-pancreatic artery is given off by the hepatic as it passes the pancreas.
- 69. The anterior mesenteric artery is the second branch of the cœliaco-mesenteric (61). It gives rise to two branches (70 and 72).
- 70. The splenic artery is a very short branch from the anterior mesenteric (69) arising soon after it leaves the cœliaco-mesenteric (61). It supplies the spleen.
- 71. The main portion of the anterior mesenteric is the intestinal artery, running to all portions of the small intestine along the mesentery proper. Some branches course through the mesorectum to supply the rectum. These are called the hæmorrhoidal arteries.

Drawing 5. Make a working sketch of the visceral arteries.

#### 6. THE VISCERAL VEINS

Passing from the organs to which you just traced the visceral arteries, trace the visceral veins.

- 72. From the rectum and small intestine, numerous small vessels converge in the mesentery proper to form the intestinal vein.
- 73. The splenic vein joins one of the large tributaries of the intestinal vein which collects blood from the rectum.
- 74. The gastroduodenal vein carries blood from the duodenum and pyloric end of the stomach into the intestinal vein, passing through the gastro-duodenal omentum.
- 75. The gastric veins from the right and left sides of the stomach join with the intestinal vein as it courses along the hepato-duodenal omentum, to form
- 76. the hepato-portal vein, which enters the liver. It is largely obscured by the pancreas through which it passes at this time. From this gland it receives numerous small pancreatic veins, too small for practical demonstration.
- 77. From the point where the hepato-portal vein enters the liver, trace back a small vein, the ramus descendens of the abdominal vein, which you have already identified (27). Note that two other rami are given off from the abdominal vein at this point, which enter the right and left lobes of the liver.
- 78. Trace the abdominal vein back along the ventral surface of the body wall until you find its origin, the union of two pelvic veins, one from each leg.
- 79. At the point of union of the pelvic veins locate a small vein from the bladder, the vesical vein.

80. Toward the cephalic end, at the point where the abdominal vein leaves the body wall, it is joined by a small vein from the heart, the cardiac vein.

Drawing 6. Make a working sketch of the hepatoportal system, which is composed of the portal and abdominal veins and their tributaries.

#### SECTION IV. THE UROGENITAL ORGANS

The urogenital system is a complex of two systems, the excretory (nephric) system and the reproductive (genital) system. The excretory system is composed of the excretory glands or kidneys (mesonephroi in the frog), their ducts, the ureters (mesonephric ducts in the frog), and a storage sac, the excretory bladder. The reproductive system is composed of the genital glands or gonads (ovaries or testes), and their gonoducts (mesonephric duct in the male, oviduct in the female). Both open into the cloaca.

Without cutting anything locate the following structures in

#### 1. THE FEMALE UROGENITAL SYSTEM

Lift the ovary (39) on one side; notice that it is attached to the body wall by

- 81. the mesovarium, a thin sheet of peritoneum (32).
- **82.** The **fat-body**, a mass of yellow finger-like processes, is attached to the cephalic end of the ovary.
- 83. The kidneys (mesonephroi), a pair of red lobulated glands, lie underweath the ovary and are attached also to the fat-bodies. They are covered with peritoneum which obscures them in the preserved specimen, as they are extra-peritoneal.
- 84. The ureter or mesonephric duct may be seen at the lateral margin of each kidney by carefully removing the peritoneum. It leads to the cloaca.

- 85. The adrenal gland, a yellowish wavy streak upon the ventral side of the kidney, may also be seen. It is a ductless gland.
- **86.** The two oviducts are highly convoluted white tubes. They open at the cephalic end by the
- 87. ostia tubæ abdominales, funnel-shaped apertures on the mesentery from the liver to the ventral body wall, just lateral to the root of the lung.
- 88. The oviduct is supported by a peritoneal membrane, the mesotubarium.

After completing the study of the visceral blood-vessels (page 14 ff.), slit open the cloaca and locate the openings of the excretory bladder, oviducts, and ureters.

Drawing 7. Make a drawing (x2) of the female urogenital system, showing the ovary on the left, the underlying organs on the right.

#### 2. THE MALE UROGENITAL SYSTEM

- 89. The testis is a yellow rounded body attached to the kidney (83) along its mesial edge by a membrane,
- 90. the mesorchium. At its cephalic end are fat bodies (82) similar to those in the female. Note also the adrenal gland (85) on the kidney, by tipping the testis towards the mesial line in order to expose the excretory organ.
- 91. Note the numerous, extremely small vessels passing along the mesorchium into the mesonephros. These are the vasa efferentia. Inside the mesonephros they unite with the mesonephric tubules of the kidney so that the spermatozoa may pass into the mesonephric duct (85), which, in the male, functions both as an ureter and a gonoduct.

- 92. At the points where the duets enter the cloaca, they enlarge to form seminal vesicles.<sup>1</sup>
- 93. A rudimentary oviduct 2 lies alongside of each mesonephric duct.

#### Drawing 8. Draw (x2) the male urogenital system.

Poorly developed in R. pipiens and R. catesbiana.

<sup>2</sup> Absent in R. catesbiana.

#### SECTION V. THE VASCULAR SYSTEM

The circulatory or blood-vascular system of the frog is a closed system, the blood being constantly confined within vessels. A heart, divided into two atria and one ventricle, pumps the blood through these vessels. From the ventricle the blood passes into one of three pairs of arches. The first, or carotid arches, carry blood to the head; the second, or systemic arches, carry blood to the rest of the body, uniting to form the dorsal aorta; the third, or pulmocutaneous arches, carry blood to the lungs and skin to be aërated. All the arteries terminate in tiny capillaries which unite to form veins. From the head and forelimbs the blood is returned by the precaval veins to the right atrium; from the viscera to the hepatic portal vein, terminating in the liver; from the hindlegs to the hepatic portal vein by the abdominal vein, or to the kidney by the renoportal veins; from the liver and the kidney to the right atrium by the postcaval vein; and from the lungs by the pulmonary veins to the left atrium.

You have already identified the caliaco-mesenteric artery from the dorsal acrta to the viscera, and the hepatoportal veins (Drawings 5 and 6).

#### 1. THE HEART

It is desirable at this point to make use of a fresh specimen, preferably injected. Open along the mid-ventral line as before. Remove the sternum (235) so as to expose the pericardial cavity (29) completely. Raising the pericar-

dium, dissect it clear from the heart on the ventral side. Identify the following parts of the heart:

- 94. The ventricle, a thick-walled muscular sac, forms the apex of the triangular heart at its caudal end.
- 95. The bulbus cordis on the ventral surface of the heart arises from the ventricle on the right. It is separated by a slight groove from the
- 96. truncus arteriosus, a thinner walled tube assuming the form of a Y at the cephalic end of the heart. The two limbs of this Y lead to the aortic arches (p. 26).
- 97. Beneath the bulbus and truncus lie the atria, right and left, which are thin walled, distensible sacs.

Tip the heart forward and identify on the dorsal surface,

- 98. the dorsal mesocardium, a membrane which is suspended. Cut this to see
- 99. the sinus venosus which receives the blood from
- 100. the two large precaval veins from the cephalic side, and
- 101. the postcaval vein from the caudal end.
- 102. The pulmonary vein, which brings blood from the lungs, may be seen lying beside the left precaval vein. It enters the left auricle.

## Drawing 9. Sketch the heart with its vessels (x2) from both dorsal and ventral aspects.

(The following dissection of the heart should be omitted if *R. catesbiana* is not available.)

Cut across the limbs of the truncus and the caval veins and remove the heart, identifying again the structures you have previously identified.

To trace the course of the blood through the heart, place it in a dish of water, dorsal side up. Carefully remove the

dorsal wall of the sinus and wash out the blood with the blowpipe. Find

103. the sinu-auricular aperture, leading from the sinus venosus (99) into the right auricle.

Place the heart ventral side up and remove the ventral wall of the auricles, taking care not to injure the truncus. Find

104. the inter-auricular septum, separating the two auricles (atria). Note the sinu-auricular aperture in the right auricle near the septum and

105. the aperture of the pulmonary vein, near the septum of the left auricle.

Again place the heart dorsal side up and remove the dorsal surface of the ventricle, to find

106. the auriculo-ventricular aperture, divided by the inter-auricular septum, at the cephalic end towards the ventral side, and

107. the ventriculo-bulbar aperture, leading into the bulbus, also at the cephalic end, but towards the dorsal side and to the right. Note

108. the semilunar valves by which this aperture is guarded. How many are there?

Slit open the bulbus and truncus and note

109. in the bulbus the spiral valve, dividing it longitudinally.

110. In the limbs of the truncus, observe the septa which divide them into three compartments, leading to the three aortic arches on each side.

# 2. The Veins

Returning to the injected frog, identify the cut end of the postcaval vein (101) and follow this caudad, noting its tributaries as follows:

111. There are three hepatic veins from the liver. These return the blood received from the hepato-portal veins (Drawing 6).

112. There are four or five renal veins from each kidney, which return the blood received from the reno-

portal veins (115).

113. The adipose veins join the first pair of renal veins, which carry blood from the fat bodies.

114. The ovarian or spermatic veins convey blood from the ovary or testis respectively, and either join the renal veins or establish direct connections with the postcaval vein (101).

115. Note on the dorsolateral margin of each kidney the reno-portal vein. This receives branches from the body wall and the oviduct. Trace it back and note that it results from the union of

116. the sciatic vein from the dorsal surface of the thigh, and

117. the external iliac vein from its ventral surface.

Trace back the external iliac until you find its juncture with the pelvic vein (78) running to the abdominal vein. The external iliac and pelvic are

118. branches of the femoral vein, the largest vessel returning blood from the hindleg. If time permits trace back the sciatic and femoral veins and study the distribution of their tributaries in the leg.

Return to the cut ends of the precaval vein. Trace forward the right precaval vein and note the following tributaries, by whose juncture it is formed (119, 123, 125).

- 119. The external jugular vein, returning blood from the head, is formed by the union of
- 120. the lingual vein from the tongue and floor of the mouth and

- 121. the mandibular vein from the lower jaw.
- 122. The second tributary of the precava is the innominate vein formed by the union of
- 123. the internal jugular vein, from the dorsal portion of the head, and
- 124. the subscapular vein bringing blood from the dorsal surface of the shoulder girdle and arm.
- 125. The third tributary of the precava is the subclavian vein which is formed by the union of
- 126. the cutaneous vein which returns the blood distributed by the cutaneous artery, and
- 127. the brachial vein which supplies the ventral portion of the arm. If time permits, trace out the distribution of the subscapular and brachial veins.

In the course of your dissection of the precaval vein, you will note three small bodies which are endocrine (ductless) glands.

- 128. Lying next to the mesial ventral surface of the external jugular is a small reddish brown oval body, the pseudothyreoid (parathyreoid) gland.
- 129. Somewhat dorsal and cephalad of this, the larger thyreoid gland may be seen.
- 130. The thymus gland may be found by tracing the internal jugular to the caudal margin of the tympanic membrane.

If these bodies have been destroyed they may be observed later during the dissection of the muscles.

Drawing 10. Make a sketch of the veins just dissected.

### 3. The Arteries

Trace the arteries on the right side of the heart, commencing with the cut end of the truncus arteriosus. This divides

into three aortic arches (131, 135, 138). (See also p. 22.)

- 131. The most anterior of the aortic arches is the common carotid which soon divides to form
- 132. the external carotid (lingual) artery, running to the lower jaw and ventral structures of the head, and
- 133. the internal carotid artery, a larger branch, running to the dorsal side of the head.
- 134. Note, at the juncture of these arteries, an oval dilation, the carotid gland.
- 135. The most posterior of the aortic arches is the pulmocutaneous, which divides into
- 136. a pulmonary artery to the lung and
- 137. a great cutaneous artery to the skin.
- 138. The middle arch is the systemic, which bends dorsally around the pharynx to unite with its mate from the opposite to form
- 139. the dorsal aorta, lying just ventral to the vertebral column. From the systemic arches spring four branches,
- 140. the laryngeal artery, to the larynx,
- 141. the occipito-vertebral artery to the skull and vertebral column,
- 142. The œsophageal artery, to the œsophagus, and
- 143. by far the largest, the subclavian artery. This vessel extends laterally and supplies the arm. If time permits, trace its branches.

Caudad of the union of the systemic aortic arches by which the dorsal aorta is formed, note the departure of the cæliaco-mesenteric artery (61) and behind this, in sequence:

144. four to six urogenital arteries supplying the kidney, fat body, ovary or testis,

- 145. one to four lumbar arteries to the body wall,
- 146. the posterior mesenteric artery to the rectum.
- 147. At its posterior end the dorsal aorta divides into two iliac arteries. Each gives off
- 148. a hypogastric artery to the urinary bladder and body wall, and
- 149. a femoral artery to the thigh, and then continues down the leg as
- 150. the sciatic artery. Trace its branches if time permits.

# Drawing 11. Sketch the arteries you have identified.

Drawings 12 and 13. Assemble the sketches you have made of the arteries, veins, and heart (Drawings 5, 6, 9, 10, and 11). Prepare from these two diagrams, one to show the arterial circulation, the other to show the venous circulation.

# SECTION VI. THE NERVOUS SYSTEM AND SENSE ORGANS

Anatomically, the nervous system is divided into three subsystems: 1, the peripheral system, a network of nerves conveying impulses from the sense organs, and to the muscles and glands; 2, the cerebro-spinal (central) system, a central co-ordinating tube; and 3, the autonomic (sympathetic) system supplying the viscera and associated with the peripheral system. The sense organs are the nose, eyes, and ears, together with scattered sensory elements in the skin and mouth.

Place the frog on the dissecting board, ventral side up, and carefully remove the urogenital system, taking care not to injure the dorsal aorta. Note that the kidneys are retro-peritoneal, and therefore outside of the calome. Identify the following structures.

- 151. The periganglionic glands (calciferous bodies), are silvery white lymph glands surrounding the ganglia of the ten pairs of spinal nerves, which leave the vertebral column between the vertebra.
- 152. The first spinal (hypoglossal) nerve is a very small nerve, leaving the spinal cord between the first and second vertebræ, and running cephalad and ventrad to the tongue. One or two fibres bend caudad to join
- 153. the second spinal (brachial) nerve, which is very large, and leaves the vertebral column between the second and third vertebræ and runs to the arm. It is joined by

- 154. the third spinal nerve which curves cephalad to meet the second and forms the brachial plexus.
- 155. The fourth spinal nerve runs caudad at an angle of forty-five degrees to the vertebral column and supplies the body wall.
- 156. The fifth spinal nerve is parallel to the fourth.
- **157.** The **sixth spinal nerve** is similar to the fourth and fifth.
- 158. The seventh spinal nerve runs caudad, parallel to the vertebral column, and joins the eighth to take part in the formation of the lumbro-sacral (sciatic) plexus. Shortly before the point of junction, it gives off the ilio-hypogastric nerve to the body wall of the abdomen.
- 159. The eighth 'spinal nerve is parallel to the seventh but mesiad to it. After its junction with the seventh it unites with the ninth spinal nerve. At this point a crural nerve is given off to the thigh.
- 160. The ninth spinal nerve, parallel and mesiad to the the eighth, unites with the other elements of the sciatic plexus to give rise to the great sciatic nerve, the largest single nerve in the body.
- 161. The tenth spinal (coccygeal) nerve is very small. It emerges from small openings in the urostyle (231) and after receiving a branch from the ninth to form the ischio-coccygeal plexus, supplies the cloaca and bladder.

(If time permits, trace the brachial and sciatic nerves to the toes.)

162. The sympathetic trunks are longitudinal nerves of the autonomic system lying along the systemic arches and dorsal aorta. Each is connected by

<sup>&</sup>lt;sup>1</sup> Largest element of the sciatic plexus of R. catesbiana.

- 163. rami intercommunicantes with the spinal nerves.
- 164. The splanchnic nerve runs along the coeliacomesenteric artery (61) and receives branches from the third, fourth, fifth, and sixth sympathetic ganglia. which unite with each other to form the solar plexus.

Drawing 14. Draw the ventral surface of the dorsal body wall, labeling all the structures you have just identified (x2).

Place the frog dorsal side uppermost. Make a transverse incision in the skin across the head just cephalad of the arms, strip the skin forward, and note:

- 165. The slender columella is in the aperture lying beneath the tympanum (6). Note its relation to the tympanum.
- 166. There is a tympanic cavity (middle ear).

Carefully remove an eyeball, by cutting through the eyelids, the muscles of the eyeball, and the optic nerve (193). Cut the eye into two portions, by cutting across it with scissors to one side of the pupil in any except the sagittal (vertical longitudinal) plane. Place the larger portion in a dish of water and note the following structures.

- 167. The transparent cornea at the external (exposed) surface above the iris, continues into
- 168. the inner opaque sclerotic layer.
- 169. The thin black pigmented chorioid layer extends outward under the cornea as the iris (3).
- 170. A thin, yellow-white layer at the proximal half of the eye is the sensory retina.
- 171. The crystalline lens is a large solid body which is transparent in a fresh specimen.

Drawing 15. Make a diagram of the eye on the basis of this dissection (x2).

Remove the muscles from the back of the head. Bend the head ventrally until a space is formed between the skull and the vertebral column. It exposes a dark membrane. Remove this with forceps and the white brain will be seen beneath it. Make two longitudinal cuts with scissors through the dorsal surface of the skull, taking great care to cut upward so as not to injure the brain. Slowly whittle away the skull roof until the brain is exposed. Do not attempt to remove it at this time. Identify the following parts from the dorsal side.

- 172. The olfactory lobes (rhinencephalon) are at the level of the caudal margins of the eyes. They give off the olfactory (first cranial) nerves (181) from their cephalic ends.
- 173. The cerebral hemispheres (telencephalon) are separated from the olfactory lobes by a shallow groove. They are separated from each other by a sagittal fissure.
- 174. The diencephalon lies caudal and a little ventral to the telencephalon. At its anterior end is a small cavity, covered, in life, by the anterior chorioid plexus which may not appear in a preserved specimen. Behind this, in exceptionally good dissections, may be seen
- 175. a small, dark, vascular body, the paraphysis, and
- 176. a thin, small tube, the epiphysis (5).
- 177. Caudal to the diencephalon and rising dorsally, are the optic lobes (mesencephalon).
- 178. Caudal to this and at a more ventral level, is a small transverse fold, the cerebellum (metencephalon).
- 179. The most caudal division of the brain is the medulla oblongata (myelencephalon), which narrows at its caudal end to join the spinal cord (180). Its roof

- is thin and covered by the posterior chorioid plexus which usually comes off with the overlying bone, to expose the fourth ventricle (201).
- 180. The spinal cord should be traced back as far as the first spinal nerve (152).

The cranial nerves of the frog are small and the dissection of them is difficult. Identify the following nerves from the dorsal aspect.

- 181. The olfactory nerve (I) arises from the olfactory lobe and runs to the nose.
- 182. The trochlear nerve (IV), very small, arises from the brain between the optic lobes and the cerebellum. (It supplies one of the eye muscles.)
- 183. The trigeminal nerve (V) arises from the medulla at its cephalo-lateral angle and passes into the proötic ganglion, where it is joined by
- 184. the facial nerve (VII).

From the prootic ganglion trace forward five branches.

- 185. The caudal branch is the hyomandibular ramus of the VIIth.
- 186. Anterior to this the maxillo-mandibular ramus of the Vth arises.
- 187. Next the temporal ramus of the Vth is given off.
- 188. Ventral to these is the ophthalmic ramus of the Vth.
- 189. Ventral to the hyomandibular ramus, the palatine ramus of the VIIth takes its origin.
- 190. The auditory nerve (VIII) leaves the brain close behind the VIIth and passes directly to the ear.
- 191. The glossopharyngeal nerve (IX) arises from the sides of the medulla by four roots which it possesses in common with the tenth cranial nerve, with which it is associated.

192. The vagus nerve (X) is associated with the IXth, along with which it leaves the skull. Outside the skull they expand to form the jugular ganglion and from this they emerge as separate nerves.

Drawing 16. Draw the brain and cranial nerves from the dorsal aspect (x2).

Remove the brain by cutting through the cranial nerves, commencing with the olfactory, and place the brain ventral side up. Identify the following structures.

- 193. The optic nerves (II) unite in the optic chiasma.
- 194. The infundibulum is a bilobed projection, often removed in dissecting, caudal to the chiasma.
- 195. Attached to the distal end of the infundibulum, and caudal to it, is the somewhat trilobed pituitary gland, another endocrine organ.
- 196. The oculo-motor nerves (III) arise dorsal to the lobes of the infundibulum and supply certain muscles of the eyeball.
- 197. Close to the mid-line of the medulla are the small abducens nerves (VI), each of which after sending a twig to the proötic ganglion proceeds to one of the muscles of the eyeball.

Drawing 17. Sketch the ventral surface of the brain indicating the nerves that arise from it (x2).

With a sharp scalpel remove the roof of the brain, noting the following cavities.

- 198. In the telencephalon, the lateral ventricles connected by the foramen of Monro make up the telocele. These are the first and second ventricles.
- 199. The foramen communicates with the third ventricle or diacœle in the diencephalon.

- 200. In the mesencephalon, the mesocœle consists of the cavities of the optic lobes and the iter or aqueduct into which they open. This passage connects the diacœle with
- 201. the fourth ventricle or myelocœle, already noted (179). This cavity narrows into
- 202. the central canal of the spinal cord.

Remove the roof of the auditory capsule (225) and note:

- 203. the membranous labyrinth of the inner ear. Unusually favorable dissections reveal
- 204. the dorsal utriculus with its
- 205. three semicircular canals, each with its ampulla,
- 206. and the ventral sacculus, with
- 207. a delicate tube, the ductus endolymphaticus, running dorsad from the cephalic margin.

# SECTION VII. THE SKELETON

If a prepared skeleton is not available, skin a frog and remove the viscera and as much of the musculature as possible. Boil for five minutes (repeated if necessary) in soap solution (see Appendix), and pick away the remainder of the soft parts.

The skeleton of the frog is entirely an endoskeleton. It is composed of the axial skeleton, including the skull, vertebræ and sternum; and the appendicular skeleton, consisting of the pectoral and pelvic girdles and the bones of the limbs.

The skull consists of certain derm-bones which form the dermocranium (D), axial elements which form the neurocranium (N), and derivatives of the gill arches (visceral skeleton) which form the splanchnocranium (S).

In the skull identify the following bones from the dorsal aspect, commencing at the cephalic end.

- 208. The premaxillaries (D) are the most anterior bones bearing teeth.
- 209. The maxillaries (D) also bear teeth and form the greater part of the upper jaw.
- 210. Articulating with the premaxillaries at their caudal ends are the nasals (D), which send long processes laterad to join the maxillaries.
- 211. Caudal to the nasals, and partially overlaid by them, is the sphenethmoid (N).
- 212. This is overlaid caudad by the long fronto-parietals (D).

- 213. Caudal and ventral to these are the occipitals (N), bearing the condyles by which the skull articulates with the atlas (228) of the vertebral column. Between these, on the posterior face of the skull, is the foramen magnum, the aperture by which the spinal cord leaves the skull.
- 214. Laterad to the occipitals and articulating with the fronto-parietals are the proötics (N) which cover the inner ear. Note the opening, fenestra ovalis, covered in life by a cartilage, with which the columella (165) articulates.
- 215. Laterad to the proötics are the large T-shaped squamosals (tympanics) (D).
- 216. At the caudal lateral margins of the squamosals are the quadrato-jugals (D), which articulate with the maxillaries. They form the suspension for the lower jaw.

Drawing 18. Draw the right half of the skull from the dorsal aspect (x4).

Place the skull ventral side up. Examine the lower jaw and find the following bones.

- 217. At the cephalic end, corresponding to the premaxillaries, are the mento-meckelians (predentales) (D).
- 218. The long dentales (D) are just caudal to them. Note the arrangement of the teeth.
- 219. The angulare-splenials (D) articulate with the quadrato-jugals (216) by means of the quadrate cartilage (S). These derm-bones cover the Meckelian cartilages (S) of the lower jaw. Both cartilages arise from the first (mandibular) visceral arch of the embryo.

Remove the lower jaw and identify the following bones on the ventral surface of the skull:

- 220. Articulating with the premaxillaries are the tooth-bearing vomers (D).
- 221. Caudal to these are the thin splint-like palatines (D).
- 222. The longitudinal parasphenoid (D) extends caudad.
- 223. The triradiate pterygoids (S) articulate with the maxillaries and nasals cephalad, the parasphenoid mesiad, and the quadrato-jugal at the angle of the jaw.

In a wet specimen note also the following cartilages from which the derm-bones have been removed:

- 224. The nasal capsule (N) surrounds the nose.
- 225. The auditory capsule (N) surrounds the ear.
- 225. The subocular arch (N) connects the nasal and auditory capsules with the quadrate cartilage (219).
- 227. The hyoid cartilage (S) supports the tongue.

# Drawing 19. Draw the left half of the skull from the ventral aspect (x2).

Examine the vertebral column, consisting of ten bones.

- 228. The atlas supports the skull. This is the only cervical vertebra the frogs possess.
- 229. There are seven abdominal vertebræ. Note the dorsal neural spines, the cephalic processes (prezygapophyses), caudal processes (postzygapophyses), and lateral transverse processes (diapophyses). The latter articulate with short cartilages sometimes considered ribs.<sup>1</sup>
- 230. The sacral vertebra has, united to it, the ilium of the pelvis (242).

<sup>&</sup>lt;sup>2</sup> See Kingsley, The Vertebrate Skeleton, p. 41.

231. The urostyle (coccyx) represents the fused caudal vertebræ. Note the small apertures by which the tenth spinal nerve emerges.

Examine the breast bone from the ventral aspect, identifying the following bones:

- 232. At the cephalic end is the episternum, with a cartilaginous plate projecting from it cephalad. At the caudal end it articulates with
- 233. two derm-bones (not part of the sternum proper), the clavicles, which run laterad to the scapulæ (236).
- 234. Caudad of the clavicles are two stout transverse bones, the coracoids, elements of the pectoral girdle.
- 235. The most caudal structure is the sternum, terminating in a cartilaginous plate.

Examine the pectoral appendage, shoulder and arm, and identify the following bones. The girdle consists of the coracoid (234), and a cartilaginous procoracoid partially covered by the clavicle (233), and two other elements (236 and 237).

- 236. The scapula is a lateral element bearing on its lateral margin a notch, the glenoid fossa, the remainder of which is formed by the coracoid.
- 237. The dorsal bone is the suprascapula.

The free appendage contains twenty-three bones; identify the following:

- 238. The proximal bone is the humerus, fitting into the glenoid fossa.
- 239. Distal to this is the radio-ulna, resulting from the fusion of the radius and ulna of the embryo.
- 240. This is followed by the carpus or wrist containing

six bones, the proximal ulnare, centrale and radiale, and the distal carpales.

241. The distal group of bones is the manus or hand, containing the five metacarpales and the phalanges, two for each of the inner digits, three for the outer digits, fifteen bones in all.

Examine the pelvic appendage (pelvis and leg) and identify the following bones:

The girdle consists of:

- 242. the long, sword-shaped ilium, articulating at its cephalic end with the ninth vertebra.
- 243. the ischium, caudal to the illium, and
- 244. the pubis, ventral to the two others. The acetabulum, or cup into which fits the head of the femur (245), is formed by the union of these three bones.

The free appendage contains twenty-eight bones.

- 245. The proximal bone is the femur, its head fitting into the acetabulum.
- 246. Distal to this is the tibio-fibula, resulting from the fusion of the embryonic tibia and fibula.
- 247. This is followed by the tarsus or ankle containing five bones, the tibiale, fibulare, centrale (at the base of the prehallux (10), and two tarsales.
- 248. The distal group of bones is the pes or foot, containing two small bones of the prehallux, five metatarsales, and the phalanges, two in the first and second digits, three in the third and fourth digits, and four in the fourth digit, twenty-one bones in all.

<sup>&</sup>lt;sup>1</sup> The prehallux may have from one to three elements.

#### SECTION VIII. THE MUSCULATURE

For the study of the musculature in detail a fresh specimen of R. catesbiana is desirable. If this is not feasible, the dissection of the muscles of the leg (pp. 44-6) at least should be completed, using the material already at hand. The muscular system includes not only the muscles attached to the skeleton but also the muscles of the viscera, skin, and sense organs. The skeletal muscles alone will be considered in this guide, and only the more prominent of these. The advanced student is referred to Gaupp, where over one hundred and fifty separate skeletal muscles are described. In the description of the muscles, the term origin refers to the relatively immovable attachment of the muscle, the term insertion to the part moved. In dissecting the muscles, they should be spread apart with the handle of the scalpel. When the directions call for the cutting of a muscle, this should be done by lifting it carefully and making an incision through the middle (belly) of the muscle from below.

Skin a frog, exercising great care not to tear any of the underlying structures. Place it on the dissecting board and identify the following muscles, commencing at the cephalic end. Sketch as you dissect, showing the superficial muscles at the right of the mid-line of your drawing, and the deeper muscles at the left. Do not attempt to verify the insertions and origins until you have identified the adjacent muscles.

- 249. Temporalis. In front of the tympanum. Origin from proötic and occipital: insertion on angulare.
- 250. Depressor mandibulæ. Behind tympanum. Ori-

- gin from dorsal fascia (connective tissue along midline of the back): insertion on lower jaw.
- 251. Dorsalis scapulæ. Covering suprascapula. Origin from suprascapula: insertion on humerus.
- 252. Latissimus dorsi. Caudal and dorsal to dorsalis scapulæ. Origin from dorsal fascia: insertion on humerus.
- 253. Longissimus dorsi. A longitudinal muscle, caudal and ventral to the latissimus. Origin from urostyle: insertion on occipitals. It is marked by a number of transverse white lines, the septa tendinea.
- 254. Coccygeo-sacralis. At the lateral margin of the caudal end of the longissimus. Origin from urostyle: insertion on sacral vertebra.
- 255. Coccygeo-iliacus. Caudal and lateral to coccygeo-sacralis by which it is covered cephalad. Origin from urostyle: insertion on ileum.
- 256. Obliquus externus. The broad lateral muscle of the body wall. Origin from dorsal fascia: insertion on ventral aponeurosis (corresponding to dorsal fascia on ventral side). One portion (pars scapularis) at the cephalic end connects the suprascapula with the sternum.

Cut through the obliquus externus and note ventral to it:

257. Transversus, a similar sheet, but with its fibers at a different angle. Origin from ileum, fascia dorsalis and the fourth vertebra: insertion on ventral aponeurosis.

Carefully pull the depressor mandibulæ cephalad and lift suprascapula exposing the following muscles:

258. Rhomboideus anterior, at the cephalo-mesial border.
Origin from dorsal fascia and fronto-parietals: insertion on ventral surface of suprascapula.

- 259. Levator scapulæ superior, lateral to the rhomboideus anterior. Origin from the proötic, and squamosal: insertion on ventral surface of suprascapula.
- **260.** Levator scapulæ inferior, lateral and ventral of the rhomboideus anterior. Origin from occipital: insertion on ventral surface of suprascapula.
- 261. Cucularis, at the lateral margin, a long thin muscle. Origin from proötic and occipital: insertion on cephalic margin of scapula. Note between the cucularis and the depressor mandibulæ the thymus gland (130).

Pull the latissimus dorsi caudad and lift suprascapula, exposing

- 262. Rhomboideus posterior at the mesial border. Origin from 3d and 4th vertebræ: insertion on suprascapula.
- 263. The serrati, three small muscles arising from the 3d and 4th vertebre, two of which insert on the suprascapula, and the third on the scapula.

Drawing 20. Complete sketch of dorsal body musculature (x2).

Turn the frog ventral side uppermost and identify the following muscles, commencing a sketch as before:

- 264. The submaxillaris, uniting the halves of the lower jaw.
- 265. Subhyoideus, immediately caudad. Origin from the hyoid: insertion on lower jaw.
- 266. Extending caudad from beneath the submaxillaris, the geniohyoideus on each side of the mid-line. Origin from lower jaw: insertion on hyoid.

- 267. Between and dorsal to these, the hyoglossus. Origin from hyoid: insertion in tongue.
- 268. Laterad, the deltoideus passes to the arm. It has three portions originating from the sternum, clavicle and scapula respectively and inserted on the humerus.
- 269. Overlying this and caudad, the great pectoralis extends from the sternum to the humerus. It has three origins, the epicoracoid, sternum, and the sheath of the rectus abdominis (270).
- 270. Caudad to the pectoralis, the rectus abdominis extends longitudinally on each side of the mid-line from the pubis to the sternum. Note the white transverse tendonous inscriptions. At the sides the obliquus externus (256) may be seen.

Pull the deltoideus cephalad and the pectoralis caudad, exposing between them,

271. the coraco-radialis, with its broad origin along the episternum and insertion on the radial portion of the radio-ulna.

Cut through the deltoideus and the pectoralis and identify:

- 272. the coraco-brachiales, two slender transverse muscles. Origin from coracoid: insertion on humerus.
- 273. Anconeus (triceps). This great muscle of the upper arm has one head originating from the scapula and three from the humerus. The insertion is on the ulnar side of the radio-ulna. (The dissection of the muscles of the lower arm and hand are omitted in this manual.)

Cut through the submaxillaries in the median line, exposing 274. the submentalis, uniting the tips of the lower jaws.

Retract the sternum, exposing

- 275. the sternohyoideus with origin from the sternum and insertion on the hyoid.
- 276. The omohyoideus with its origin on the scapula and insertion on the hyoid. In the angle formed by these muscles as they converge towards the hyoid, note the parathyreoids (128).

Cut through the sternohyoideus and observe:

277. the petrohyoidei, four small muscles from the auditory capsule (225) to hyoid. Note in the notches of the hyoid, close to the origin of the hyoglossals, the thyreoid glands (129).

Open the jaws widely, remove the skin from the mouth and identify the temporalis (249) and the following:

- 278. pterygoideus. Cephalad and mesiad of the temporalis. Origin from the fronto-parietal and proötic: insertion on lower jaw.
- 279. The masseters, two small muscles of which one runs from the cephalic border of the tympanum to the angulare, concealing the other which connects the quadrato-jugal with the angulare.

Drawing 21. Complete drawing of ventral body musculature (x2).

## THE MUSCLES OF THE HIND LEG

Place the frog ventral side up and identify the following muscles, making a sketch as you proceed.

In the thigh, commencing at the lateral margin:

280. Triceps femoris. This great muscle has three origins; the caput anticum from the cephalic margin of the acetabulum (244), caput medium from the ilium, and caput posticum from the same bone at the caudal end: insertion on tibio-fibula.

- **281.** Sartorius, a flat strip of muscle, extending straight down the thigh. Origin from ilium: insertion on tibio-fibulare.
- 282. Adductor magnus, large and thick, mesial of the sartorius, beneath which it passes distally. Origin from pubis and ischium: insertion on femur.
- 283. Gracilis (rectus internus) major, a large muscle at the mesial border of the thigh. Origin from ischium: insertion on tibio-fibula.
- 284. Gracilis (rectus internus) minor, a small thin muscle, forming the mesial margin of the thigh. Origin from ischium: insertion with that of the gracilis major.

In the shank commencing at the lateral margin:

- 285. The tibialis anticus longus. Origin from femur: insertion on tibiale and fibulare.
- 286. Extensor cruris, a slender muscle lying close to the tibio-fibula. Origin from femur: insertion on tibio-fibula.
- 287. Tibialis anticus brevis, very short. Origin from tibio-fibula: insertion on tibiale.
- 288. Tibialis posticus, a long thin muscle lying close to the mesial side of the tibio-fibulare from which it takes its origin: insertion on tibiale.
- 289. Plantar longus (gastrochemius), the largest leg muscle, at the mesial margin. Origin from two heads, one from a tendon connecting femur and tibio-fibula, the other from tendon of triceps: insertion in a broad tendon (aponeurosis) on the sole of the foot.

Drawing 22. Draw the ventral musculature of the hind leg (x2).

From the dorsal aspect, identify the following muscles. Sketch.

In the thigh, commencing at the lateral margin:

- 290. At the proximal end the small iliacus externus. Origin from ilium: insertion on femur. At its distal end it is obscured by the caput posticum of the triceps (280).
- 291. From the urostyle another small muscle, the pyriformis, extends to the head of the femur.
- fibularis. Origin from ilium: insertion on tibiofibula.
  - 293. The large muscle at the mesial margin is the semimembranosus. Origin from ischium: insertion on tibio-fibula.

In the shank, in addition to the plantar longus (289) and tibialis anticus longus (285), note

294. the peroneus, between the two just noted. Origin from femur: insertion on fibia and fibulare.

Cut through the sartorius (281) and identify:

- 295. The adductor longus, a narrow muscle covered by the sartorius. Origin from the ilium: insertion with that of adductor magnus (282).
- 296. The iliacus internus may now be identified. It extends from the ventral border of the ilium to the femur.

Cut through the gracilis major (283) and identify

297. the semitendinosus, a delicate muscle with two heads, covered by the gracilis major. Origin from ischium: insertion on tibio-fibulare.

The deeper muscles of the thigh, six in number, as well as the muscles of the foot, are omitted.

Drawing 23. Draw the dorsal musculature of the hind leg (x2).

# SECTION IX. HISTOLOGY

The slides loaned you have been prepared by preserving the tissues, cutting them in thin slices (sections) (except the blood, which has been smeared on the slide) dyeing them in contrasting colors, and mounting them in a transparent resin under the coverslip. By careful study you can identify on them examples of the principal tissues of the vertebrate body.

#### 1. BLOOD

In the slide given you observe

- 298. the erythrocytes (red blood corpuscles). These are elliptical cells with deeply staining oval nuclei.
- 299. The leucocytes (white blood corpuscles). Much fewer in number, these may be recognized by their irregular shape, large granular and often irregularly shaped nucleus and the occasional presence of granules in the cytoplasm.
- 300. The blood-plasma is represented by the faintly staining background between the cells. Some slides may show a few twisted elongated cells with small nuclei and flagella. These are parasitic protozoa, *Trypanosoma ranarum*.

Drawing 24. Draw examples of erythrocytes and leucocytes highly magnified.

# 2. CARTILAGE (HYALINE)

In the slide provided you, identify

301. the cartilage cells, small, ovoid and often associated in groups of two, four, etc. Study under high power.

- 302. The matrix, a lightly stained mass (transparent in life) with spaces, lacunæ, in which the cells appear.
- 303. The perichondrium, a layer of connective tissue with small blood vessels, covering the cartilage.

Drawing 25. Draw a section showing all structures mentioned above.

- 3. Bone (Transverse Section of Femur, Decalcified)
  In the slide provided, note the following:
  - 304. The periosteum, a layer of connective tissue, resembling the perichondrium (303).
  - 305. The outer zone of compact bone. Study with high power and observe
  - 306 the irregularly star-shaped bone cells.
  - 307. The matrix with its concentric layers, the laminæ, the lacunæ surrounding the cells, the widely branching delicate canals leading from them, the canaliculi. Sometimes the cells are grouped around cavities containing blood vessels and nerves. These cavities are called Haversian canals.
  - 308. The bone marrow, forming the center of the femur. It is essentially an adipose connective tissue with many blood corpuscles. Examine under high power.

Drawing 26. Draw in outline the entire section. Fill in the details in a narrow sector only.

4. STRIATED MUSCLE. (LONGITUDINAL SECTION)

In the slide loaned you, identify:

309. The muscle cells or fibers. They are grouped in bundles or fasciculi. Study under high power and note in each fiber several nuclei, longitudinal striations (sarcostyles), transverse striations (dividing

the fiber into sarcomeres), and the delicate cell membrane (sarcolemma).

310. The connective tissue cells. Inside the fasciculus they are called endomysium, around the fasciculi they are known as perimysium, while outside the muscle they form the epimysium.

Drawing 27. Sketch a section of the muscle and a detail of one fiber.

# 5. Nerve. (Cross-section of Sciatic Nerve)

In the sections of the nerve, observe:

- 311. The individual nerve fibers. Unlike the muscle fibers each is composed of more than a single cell. In the individual fiber observe under high power
- 312. the axis cylinder. Under the highest powers fine dots, cross-sections of fibrils, appear. The fiber is surrounded by a delicate exolemma. The nuclei are in the spinal cord or ganglia.
- 313. The medullary sheath surrounds the axis cylinder.
- 314. The sheath is in turn surrounded by a delicate membrane, the neurolemma. The nuclei of the neurolemma are small and compact.
- 315. Around the nerve is a layer of connective tissue, the perineurium.

Drawing 28. Draw a sector of the nerve under high power.

#### 6. THE SKIN

In the section of the skin, identify the following structures.

- 316. The epidermis, stratified epithelium. It consists of two layers,
- 317. the stratum corneum, squamous epithelium, at the outer surface, and

- 318. the stratum germinativum (Malpighii), columnar epithelium, below the corneum.
- 319. The dermis (corium) underlies the epidermis and is connective tissue. Like the epidermis it is divided into two layers,
- 320. the stratum spongiosum, towards the exterior, which is a loose layer containing the dermal glands (323), and the pigment cells (chromatophores), and
- 321. the stratum compactum beneath containing both white and yellow fibers, some smooth muscle cells, blood vessels and nerves.
- 322. Beneath the dermis is the sub-cutaneous layer of connective tissue which connects the skin with the muscles of the body wall. This is a loose connective tissue, divided into two layers by the lymph spaces.
- 323. The glands are of two types, the more frequent and smaller mucous glands and the relatively rarer and larger poison glands. The glands are composed of a layer of epithelial secretory cells, surrounded by a layer of smooth muscle cells, which in turn is covered with fibrous connective tissue. Each gland communicates with the exterior by a duct whose aperture is called a stoma.

Drawing 29. Draw a section of skin to show as many of these structures as possible.

7. THE INTESTINE (TRANSVERSE SECTION OF ILEUM)

In the section provided, locate the following structures:

- 324. The interior of the tube is lined by the mucosa, which is secretory columnar epithelium. Under high power distinguish the goblet cells which have an oval vacuole filled with mucus at the inner end.
- 325. Exterior to the mucosa lies the submucosa, an ex-

- cellent example of areolar connective tissue, with fibers, blood vessels and lymph spaces.
- 326. Outside the submucosa is a thick layer of circular smooth muscles and
- 327. a thin layer of longitudinal smooth muscles.
- 328. The exterior layer is a thin covering of fibrous connective tissue and of squamous epithelium, the peritoneum. Many capillaries may be seen.

Drawing 30. Draw an outline of the intestine, filling in the details in one sector only.

### SECTION X. EMBRYOLOGY

Within the limits of this manual, it is possible to give directions for a rapid survey of the principal external features only of the development of the frog. This survey can be performed most advantageously in the spring when living material is available. But as this is not always feasible, the directions are based on preserved material. This will be given you in watch-glasses to be studied with the aid of a hand-lens. Be careful in handling the material. When you desire to change the position of an egg or embryo do so by using a gentle stream of water from a pipette.

#### 1. CLEAVAGE AND GERM LAYER FORMATION

Examine the following stages.

- 329. The fertilized egg before cleavage (one-cell stage). Note particularly: the egg-envelope (jelly), the apical (animal) hemisphere with its black pigment, the white abapical (vegetative) hemisphere. It is sometimes possible with fresh material to see the small polar bodies at the apical pole, and the gray crescent between the apical and abapical hemispheres. (Demonstration of spermatozoa may be shown at this time.)
  - **330.** The first cleavage (two-cell stage). Note the first cleavage furrow. What relationship has it to the apical pole (and the gray crescent, if present)?
  - 331. The second cleavage (four-cell stage). Note the first two cleavage furrows. What is their relation-

ship to the structures already noted and to each other? Note that the furrows do not form a right angle at the apical pole; the small cross furrow is called the polar furrow. How is it produced?

332. The third cleavage (eight-cell stage). Note the third, meridional, cleavage furrow. What relationship has it to the first two and to the hemispheres? Which of the cells (blastomeres) are the larger?

333. The fourth cleavage (twelve- and sixteen-cell stages). Note that the fourth cleavage is produced by two furrows. What is their relationship to each other, and to the other cleavage furrows? How can you account for the existence of the twelve-cell stage?

334. The fifth cleavage (twenty-four and thirty-two cell stages). The fifth cleavage is also produced by two cleavage furrows. How does their relationship to each other differ from that of the two furrows in the fourth cleavage? How can you account for the existence of a twenty-four cell stage? Are the blastomeres equal in size and regular in arrangement, or not?

335. The blastula. Note the small size of the cells. Hemisect through the apical pole and note the cavity (blastocœle). Examine under binocular microscope if possible. If blastulae are not available, this exercise can be performed on the fifth cleavage stage (334). The cavity is then called the segmentation cavity.

336. A late gastrula (yolk-plug stage). Note the small white protuberant yolk-plug, which occupies the potential blastopore. All the rest of the surface is composed of minute pigmented cells, the ectoderm. Hemisect the gastrula through the yolk-plug and

observe a small excentric cavity, the gastrocœle. It is lined with minute white cells, the endoderm. The third germ layer, the mesoderm, may be shown in demonstration by prepared sections.

Drawing 31. Draw these stages including the hemisections (9) in such a way as to show the greatest number of structures on each. Make each drawing about two inches in diameter.

# 2. EARLY EMBRYOS AND LARVÆ

Examine the following stages:

- 337. An early embryo (neural groove stage). Note on the dorsal aspect the neural folds, meeting at the cephalic end, and approximating each other at the caudal end. Between the neural folds is the broad neural plate. The blastopore is a narrow slit between the caudal ends of the neural folds. What has become of the yolk-plug?
- 338. An early embryo (neural tube stage, 3 mm. long). The neural folds have now united to form a neural tube. Note in the head region, the stomodæum (the future mouth), a narrow slit on the ventral surface, enclosed by ridges which will become the jaws. The beginnings (primordia) of the oral suckers may be seen ventral to the stomodæum. On each side of the head is a bulge caused by the developing eye. Behind this is a swelling known as the gill plate, with five furrows, the visceral (gill) grooves. Behind the last is a swelling caused by the pronephros. On the dorsal side of the body are slight furrows indicating the presence of somites. A ventral bulge is the yolk-sac. There is a slight tail bud, beneath which is a small vent.

339. The larva at hatching 1 (6 mm.). Note the changes that have taken place since the 3 mm. stage in the structures mentioned above. On the third and fourth visceral (gill) arches, external gills have appeared. Dorsal and lateral to the margins of the stomodæum are two minute olfactory pits which are the beginnings of the nose.

Drawing 32. Draw these three stages one above the other, from the left side, magnified about twenty times.

3. The Dissection of the Tadpole (Rana catesbiana)

The bullfrog spends the first two years of its existence in the larval or tadpole stage. The large size of the tadpole offers an excellent opportunity for a dissection of the larval organs and a comparison with those of the adult. Place the tadpole on its right side and observe:

340. the external characters. What changes have taken place since hatching? Find the ventral mouth, armed with horny teeth; the tiny nostrils, one about a quarter of an inch dorsal of each side of the mouth; the eyes; the opercular aperture half way down the trunk on the left side; the tail, with its fin; and at the base of the latter locate the tiny hind limbs. The vent is at the base of the fin on the right side.

Drawing 33. Make a drawing of the tadpole from the left side. Place the tadpole on its back and remove the body wall from the ventral surface, taking care not to injure the opercular aperture.

<sup>&</sup>lt;sup>1</sup>The larva of *R. pipiens* under laboratory conditions varies in length from 3 to 6 mm. at the time of hatching.

341. Note the heart in its pericardial cavity. On either side find the internal gills, in the opercular cavity. Probe through the opercular aperture. Count the gills, and note the gill (visceral) clefts between them. Probe through the mouth. Deep in the caudolateral angles of the opercular cavity find the diminutive forelimbs. In the visceral cavity note the long coiled intestine. Lay it to one side after cutting through the rectum at the caudal end and the dorsal mesentery by which it is attached to the body wall. Do not cut through the cephalic end. At the cephalic end, note the stomach and slender vellow pancreas, the liver and gall bladder, and the esophagus. Compare with the digestive system of the adult. Find the lungs. On the dorsal surface of the colome, note the conspicuous yellow fat-bodies attached to the kidneys, relatively larger than in the adult, and the rudimentary gonads.

Drawing 34. Draw (x2) from the ventral surface, showing the intestine displaced to your left, in order to expose the organs normally concealed by it.

Remove tail by a transverse incision behind the vent.

342. Examine cut surface and locate: notochord, spinal cord, caudal artery, and muscles.

Drawing 35. Draw (x4).

#### APPENDIX

The supplies for each student required to complete the directions in this manual are:

One injected frog (preferably doubly injected).

One frog, which need not be injected.

One mounted skeleton of frog. This is desirable but not

indispensable.

Seven histological slides, including one each of the following: blood smear; section of hyaline cartilage; transverse section of decalcified femur; longitudinal section of striated muscle; transverse section of sciatic nerve; section of skin; transverse section of ileum.

One set, ten stages in the development of the frog.

One bullfrog tadpole.

All these may be readily obtained from any of the biological supply stores. The following suggestions may be of service to the teacher who prefers to prepare his own material.

The best time to collect the frogs is at night and during the breeding season, in the Spring, when they can easily be netted

with the aid of a lantern or electric torch.

They may be kept in large tanks with from one to two inches of water at the bottom, which should be changed frequently. Overcrowding the tanks should be avoided. Frogs need not be fed during the winter months if kept in a cool place. If feeding is to be demonstrated, it should be remembered that the frog will take only moving food.

The best method of killing the frogs is by the fumes of ether. When the tissues are to be preserved for microscopic preparation, the preferable method is that of "pithing." Locate with the fingernail the point at which the skull joins the vertebral column, press the head down sharply and make a narrow, deep, transverse incision at the point located. This severs the spinal cord, and if done neatly, results in practically no loss of blood.

To preserve the frogs, use formaldehyde in 5% aqueous solution. The commercial formaldehyde is 40% formalin. Mix 5 parts of this with 95 parts distilled water. The body cavity should be opened, a little to one side of the mid-line, to insure good preservation of the viscera.

Injected specimens may be prepared by injecting the arteries through the truncus arteriosus (single injection), the hepatoportal veins through the abdominal vein (double injection), and the remaining veins through the postcava (triple injection). The most useful mass is prepared with cornstarch as follows:

Cornstarch	4	parts	by	volume
Chloral hydrate, 2% aqueous sol	4	ш		
Alcohol 95%	1			
Color and glycerine (equal parts)	1	**		

Mix and stir, strain through cheesecloth.

For colors use vermilion for arteries, insoluble Prussian blue for hepatoportal vein, chrome yellow for postcava.

Skeletons are prepared by boiling in the following soap solution.

Soap	75	grams
Potassium nitrate	12	66
Ammonia	150	cc.
Distilled water	2000	ce.

Heat the water before adding the other ingredients. Use diluted, one part of the mixture to three parts of water.

To decalcify the skull before dissecting the brain use the following mixture:

Alcohol,	95%		 1 part	by volume
Nitric ac	id. 109	sol.	 1 "	

For the preparation of the slides called for, it will be necessary to consult the works on microscopic technique mentioned in the Introduction. The blood smears may be stained with any of the regular blood stains, although hæmatoxylin and eosin give preparations quite adequate for class use. The sections may be prepared by making use of sublimate-acetic for fixation, paraffin embedding, Delafield's hæmatoxylin and eosin as stains, and balsam for the mounting medium. For the sections of skin and intestine, the writer recommends Bouin's fixing fluid, and his own modification of Cajal's fuchsin and picro-indigo-carmine. This gives a

<sup>&</sup>lt;sup>1</sup> Vertebrate Embryology, page 283.

polychromatic stain which brings out the different layers most vividly.

Directions for the collection and preparation of frog eggs and embryos are given in the writer's *Vertebrate Embryology*, to which the reader is referred for further information (Chapter XI, *loc. cit.*)



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